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Steroid Glucosides from *Aloe barbadensis*

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Two glucosides, β-sitosterol and lupeol were isolated from the leaves of *Aloe barbadensis*. The structures of two glucosides were elucidated as to be β-sitosterol glucoside and its palmitate.

Aloe is well known as a folklore medicine, which is used as stomachic and laxative and for burns, wounds, contusions, sprains and tooth ache. Some *Aloe* species, *A. ferox* Miller, *A. africana* Miller, *A. spicata* Backer and their cross-breeds are described in Japan Pharmacopoeia XII. *Aloe arborescens* Miller, and *A. barbadensis* Miller (=*A. vera* Miller) have also been used as above in Japan.

About 250 *Aloe* species are distributed in Africa, 40 spp. in Madagascar Island, and some species in Canary Islands.

The occurrence of phenolics, anthraquinones and polysaccharides in many *Aloe* species has been reported (Suga and Hirata 1983), and we now report the isolation of a triterpene, a steroid and its two glucosides from *Aloe barbadensis*.

Experimental

Apparatus

All melting points were determined with a Yanagimoto MP micromelting point apparatus. IR was measured with a JASCO A-102 infrared spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were recorded using a JEOL GSX-400 (^{1}H 400 MHz and ^{13}C 100 MHz) spectrometer in $CDCl_3$ or C_5D_5N with tetramethylsilane (TMS) as an internal standard. The chemical shifts were expressed in ppm δ). The $[\alpha]_D$ values were determined with a JASCO DIP-140 digital polarimeter. Kieselgel 60 F_{254} (MERCK) precoated plates were employed for thin layer chromatography (TLC) and the spots were detected with dil. H_2SO_4. Column chromatography was carried out on 70–230 mesh silica gel (MERCK). High performance liquid chromatography (HPLC) was performed using an SSC 3100-J pump with an Oyo-Bunko Uvilog 7 UV detector. EIMS and FAB-MS spectra were measured by a JEOL JMS-DX 302.

Extraction and isolation of compounds I, II, III and IV

The leaves of *Aloe barbadensis*, which were freeze-dried (222 g), were extracted with $CHCl_3$ (1 day × 3 times). After removal of the solvent by evaporation, the residue (24 g) was chromatographed on a silica gel column (hexane:acetone 20:1→0:1) and then applied to HPLC (Nucleosil 50-5, eluted with $CHCl_3$:MeOH 50:1→15:1), and we obtained compound I as white powder (32 mg, 0.014%), II as white powder (41 mg, 0.018%), III as slightly yellow grease (34 mg, 0.015%) and IV as white powder (27 mg, 0.012%) (Chart. 1).

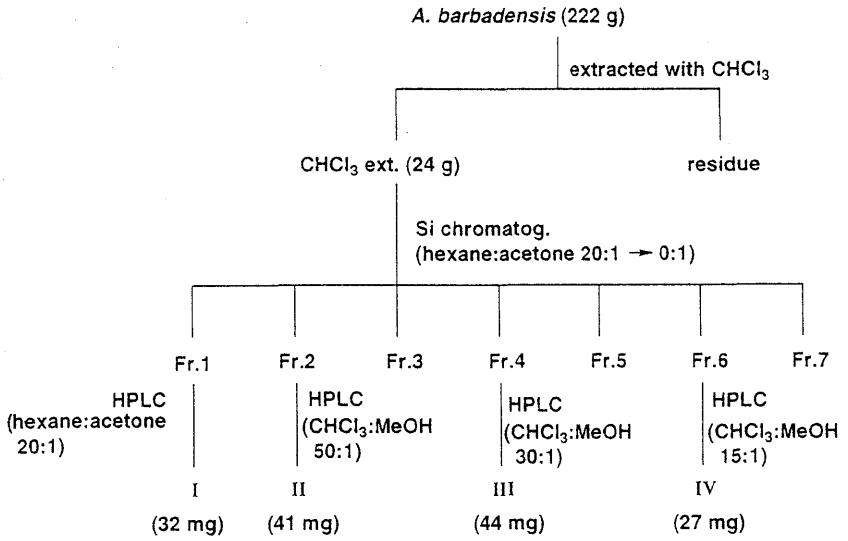

Fig. 1. Chart for extraction and isolation of compounds I, II, III and IV.

Acid hydrolysis of Compounds III and IV

Compounds III and IV (5.0 mg) were hydrolyzed with 3.5% HCl (1 ml) at 110°C for 3 hours. Then the reaction mixture was extracted with $CHCl_3$. The organic layer was washed with H_2O, dried over Na_2SO_4 and evaporated to a solid. The solid was applied to HPLC (Nucleosil 50-5, eluted with $CHCl_3$:MeOH= 50:1) to obtain an aglycone. The aglycone was identified by comparison of Rf value with that of standard β-sitosterol on TLC using a solvent system ($CHCl_3$:MeOH=15:1) to its ^{13}C-NMR spectral properties (Table 1).

Alkaline hydrolysis of compound III

Compound III (30 mg) was dissolved in EtOH (2.5 ml), and the solution was added with 15% KOH (0.5 ml) under cooling. The reaction mixture was kept at room temperature for 13 hours. After removal of EtOH, the reaction mixture was chromatographed on silica gel column (hexane:acetone 10:1→1:1), and an acid and a sterol glucoside were obtained. The acidic substance was identified as to be methyl palmitate by GC analysis. The sterol glucoside was identical with compound IV.

Identification of compounds I, II, III and IV

Lupeol (I). White powder, mp 164–165°C ($CHCl_3$-MeOH), $[\alpha]_D$ +36.9° ($CHCl_3$, c=0.79). IR ν_{max}^{KBr} cm^{-1}: 3420, 1680, 1640, 1450, 1380, 1040, 880. EIMS m/z: 426.3866 (M^+, calcd for $C_{30}H_{50}O$:426.3849). ^{1}H-NMR: δ0.76(3H, s), 0.79(3H, s), 0.83(3H, s), 0.94(3H, s), 0.97(3H, s), 1.03(3H, s), 1.68(3H, s), 3.19(1H, dd, *J*=11.2, 5.0 Hz), 4.57(1H, d, *J*=2.4 Hz), 4.69(1H, d, *J*=2.4 Hz).

^{13}C-NMR (Table 1) (Sholichin et al. 1980).

β-Sitosterol (II) was identified in comparisons with the published data of ^{1}H- and ^{13}C-NMR (Akihisa et al. 1986)

β-Sitosterol-3-*O*-β-D-(6'-palmitylglucoside) (III). Slightly yellow grease, $[\alpha]_D$-51.9° ($CHCl_3$, c=0.41). IR ν_{max}^{KBr} cm^{-1}: 3400, 2950, 2860, 1735, 1462, 1380, 1175, 1020. FAB-MS m/z: 837[M+Na]$^+$. ^{1}H-NMR($CDCl_3$): δ 0.68(3H, s, 18-H_3), 0.82(3H, t, *J*=7.2Hz, 26 or 27-H_3), 0.84(3H, d, *J*=7.2Hz, 26 or 27-H_3), 0.85(3H, t, *J*=7.4Hz, 29-H_3), 0.87(3H, t, *J*=6.9Hz, 16"-H_3), 0.92(3H, d, *J*=6.6Hz, 21-H_3), 1.01(3H, s, 19-H_3), 3.36(1H, t, *J*=8.0Hz, 2"-H), 3.38(1H, t, *J*=9.0Hz, 2'-H), 3.46(1H, m, 3-H), 3.56(1H, m, 5'-H), 3.57(1H,

Table 1. ^{13}C-NMR chemical shifts of compounds I, II, III and IV[a)]

C	I	II	III	IV
1	37.3	38.7	37.7	37.5
2	31.5	27.5	30.3	30.2
3	71.8	79.0	78.4	78.1
4	42.3	38.9	39.4	39.3
5	140.7	55.3	141.0	140.9
6	121.7	18.3	122.0	122.0
7	31.8	34.3	32.2	32.2
8	31.8	40.9	32.1	32.1
9	50.2	50.5	50.4	50.3
10	36.5	37.2	37.0	37.0
11	21.0	20.9[b)]	21.3	21.3
12	39.8	25.2	40.0	40.0
13	42.4	38.1	42.5	42.5
14	56.8	42.9	56.9	56.8
15	24.3	27.5	24.5	24.5
16	28.3	35.6	28.6	28.5
17	56.1	43.0	56.3	56.2
18	11.8	48.0	12.0	12.0
19	19.4	48.3	19.4	19.4
20	36.2	150.9	36.4	36.4
21	16.8	29.9	19.0	19.1
22	33.9	40.0	34.2	34.3
23	26.1	28.0	26.4	26.4
24	45.8	15.3	46.1	46.0
25	29.1	16.1[b)]	29.5	29.5
26	19.0	16.0[b)]	19.2	19.2
27	19.8	14.6	20.0	20.0
28	23.0	18.0	23.4	23.4
29	11.9	19.3	12.2	12.2
30		109.3		
1'			102.9	102.6
2'			75.2	75.3
3'			78.8	78.6
4'			71.7	71.7
5'			75.2	78.5
6'			64.7	62.8
1"			173.6	
2"–15"			23.0	
			25.4	
			29.5	
			29.6	
			29.7	
			30.0	
			32.1	
			34.5	
16"			14.3	

[a)]Solvent for compounds I and II: $CDCl_3$, compounds III and IV: C_5D_5N,
[b)]Assignment may be interchanged.

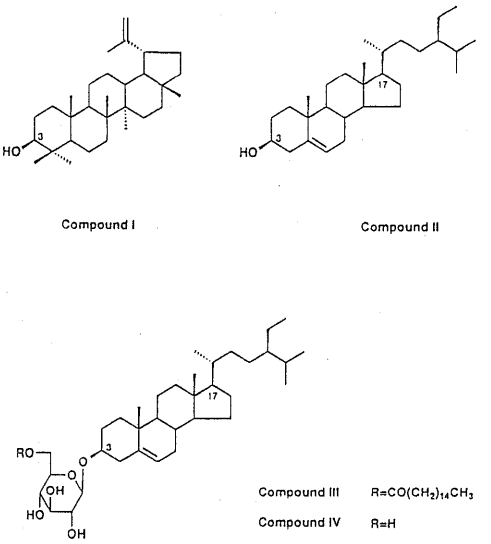

Fig. 2. Structures of Compound I, II, III and IV.

, J=9.0Hz, 3'-H), 4.26(1H, dd, J=12.0, 2.0 Hz, 6'-H), 4.38(1H, d, J=8.0Hz, 1'-H), 4.49(1H, dd, J=12.2, 4.9Hz, 6'-H), 5.36(1H, br. d, J=5.0Hz, 6-H).
^{13}C-NMR (Table 1) (Kojima et al. 1990).

β-Sitosterol-3-O-β-D-glucoside (IV). White powder, mp 259–262°C ($CHCl_3$-MeOH), $[\alpha]_D$-62.3° ($CHCl_3$, c=0.29), IR ν_{max}^{KBr} cm^{-1}: 3400, 2950, 1460, 1380, 1162, 1075, 1020. MS EIMS m/z: 576 [M$^+$]. ^{1}H-NMR(C_5D_5N): δ 0.65(3H, s, 19-H_3), 0.86(3H, d, J=7.3Hz, 26-H_3), 0.88(3H, d, J=7.7Hz, 27-H_3), 0.89(3H, t, J=7.8Hz, 29-H_3), 0.93(3H, s, 18-H_3), 0.99(3H, d, J=6.8Hz, 21-H_3), 2.46(1H, t, J=11.4Hz, 4-H), 2.72(1H, dd, J=13.5, 2.6Hz, 4-H), 3.93(1H, m, 3-H), 3.95(1H, m, 5'-H), 4.05(1H, t, J=8.0Hz, 2'-H), 4.28(2H, m, 3'-H, 4'-H), 4.40(1H, dd, J=11.9, 2.2Hz, 6'-H), 4.55(1H, dd, J=11.9, 2.2Hz, 6'-H), 5.00(1H, d, J=7.8Hz, 1'H), 5.34(1H, br.d, J=4.5Hz, 6-H).
^{13}C-NMR (Table 1) (Kadota et al. 1989).

Results and Discussion

The extract (24.0g) was chromatographed as shown in Fig. 1, and four compounds were obtained. Compound I was identified as lupeol. Compound II was

identified as β-sitosterol. Both compounds III and IV were hydrolyzed and β-sitosterol and D-glucose were obtained. Compounds III and IV showed similar ^{13}C-NMR and were assumed to be glycosides by the presence of six ^{13}C signals around δ 60–80 and δ 102. However, additional peaks were found in ^{13}C-NMR data of III near δ 30 and δ 174.4. This result suggests that compound III was β-sitosterol glucoside with higher fatty acid ester. Compound III was hydrolysed with alkali to form compound IV and a product with lower polarity. ^{13}C- and ^{1}H-NMR of the latter compound showed the typical ^{13}C and ^{1}H signal patterns of fatty acid which included one methyl, one carboxyl and many methylenes. The compound was identified with palmitic acid as the methyl ester by GC analysis. As the C-6 and C-5 carbon signals of a glucose moiety of III were shifted in comparison with those of compound IV, it was assumed that the palmitate was attached to C-6 of glucose. This was determined with COLOC method. The carbonyl carbon of palmitate correlated to methylene protons at C-6 of glucose. On the basis of these results, compound III was assigned to be β-sitosterol-3-*O*-β-D-(6'-palmitylglucoside). This is the first report on the isolation of this compound from *Aloe* species.

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木下 薫，小山清隆，高橋邦夫，野口裕希，天野實：***Aloe barbadensis* より得られたステロイドグルコシドについて**

Aloe barbadensis より1種のトリテルペンと3種のステロールを単離した．それらは，ルペオール，β-シトステロール，β-シトステロール-3-O-グルコシド及びそのパルミチン酸エステルであった．